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13. ABSTRACT *(Maximum 200 words)*

We have been using genetically predisposed mice to identify genes that can modify the risk of mammary tumor development. *Apc*^{Min} female mice develop mammary tumors with a 5% incidence by 100 days of age. Treatment of female *Min*/+ mice with ENU can increase the incidence of mammary tumors to greater than 80% within 65 days of treatment. We have characterized a congenic strain of mice that carries the ROSA26 insertion on chromosome 6, flanked by about 25 cM of DNA derived from the 129 strain. These mice are resistant to *Min*-induced mammary tumor development. In order to localize the locus or loci encoding the resistance, we have produced mice carrying subsets of the 129 derived DNA. Analysis of three such lines has indicated that a strong modifier maps within 2 cM of the ROSA26 insertion. We have identified further recombinant mice that should help to determine whether the modifier is separable from the ROSA26 insertion. We are also developing mice congenic for this region from the 129 strain that do not carry the ROSA26 insertion to allow us to determine whether the modifier is separable from ROSA26.

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____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Anne R Mason 5/26/99

PI - Signature Date

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INTRODUCTION

Progress is being made at identifying genes that are responsible for many cases of hereditary breast cancer. However, the penetrance and time of tumor development can be variable in these families. The effects of background genes on the development of cancer need to be characterized. We propose to use genetically predisposed mice to identify genes that can modify the risk of mammary tumor development. Apc^{Min} female mice develop mammary tumors with a 5% incidence by 100 days of age. Treatment of female *Min/+* mice with ENU can increase the incidence of mammary tumors to greater than 80% within 65 days of treatment. We have found that the 129/Sv strain carries alleles that confer resistance to mammary tumors and have identified the chromosomal location of one of the modifier loci. We have identified a congenic strain of mice that carries a 129 allele of the modifier on the B6 background. Using these mice as a starting point, we plan to:

1. Work toward molecular identification of the mammary modifier. By first minimizing the region containing the mammary modifier through genetic recombination, and second, by identifying and testing candidate loci mapping to the region.

2. Test for loss of heterozygosity in the modifier region in mammary tumors.

This project represents a novel approach to the identification of genes controlling breast cancer development. It utilizes the combination of mice predisposed to mammary tumor development and the genetic variability existing in the inbred strains of mice.

BODY

I have listed below each of the tasks outlined in the Statement of Work from the original grant proposal. After each task the progress made has been described.

Aim 1:

Task 1: Production and identification of 20 male mice carrying recombinant chromosomes. Months 1-12; Mice: 12 ROSA26/+ males, about 50-60 B6 females, 20-30 recombinant males weaned.

Progress: The goal of this task is to identify mice carrying recombinant chromosomes to sub-divide the congenic region on chromosome 6. To do this we have crossed B6 females with B6.ROSA26/+ males, the progeny are tested with markers throughout the region to test for the presence of recombinant chromosomes. We have produced and screened over 800 progeny and have identified mice carrying the 6 recombinant chromosomes shown in Figure 1. We expected to identify more recombinant progeny, based on the published map distances between the markers, but have found fewer recombinants than expected. This may indicate that the markers flanking the R26 insertion are closer than indicated in the published maps.

We are also trying to identify mice that are carrying a portion of the Rec2 chromosome. This line was chosen because testing indicates that the modifier is carried within this region. This will allow us to further subdivide the interval and more closely map the modifier locus.

To further characterize the recombinant chromosomes, we have identified a further 12 PCR-based markers in this region of chromosome 6 that are polymorphic between B6 and 129 mice. These markers have been mapped using the recombinant chromosomes and the DNA from mice from a backcross between 129 and B6. We now have on hand several markers that map within 1 cM on either side of the ROSA26 insertion. This will allow us to further subdivide the region. Marker *D6Mit106* is the first marker proximal or the ROSA26 insertion, and D6Mit55 is the first marker distal to the insertion. The recombination events in Rec1 and Rec2 are between *D6Mit106* and ROSA26. Until further markers are identified, we cannot further define the ends of those recombinant chromosomes.

Task 2: Production of female mice carrying each recombinant chromosome. Months 3-18; Mice: 20 recombinant males (produced in Task 1), 120 B6 females, about 200 recombinant females weaned. As recombinant males are identified in Task 1, they will be mated with B6 females to produce the female mice to be used to produce the mice used in Task 3.

Progress: The goal of this task is to produce female mice carrying each of the recombinant chromosomes identified in Task 1. These females are needed to cross with *Min*/+ males to produce the mice for testing. Each of the recombinants identified in Task 1 has been crossed with B6 mice to generate the recombinant females. This goal has been accomplished for the first three recombinants identified (Rec1, 2 and 3). The second three recombinants are currently being expanded. The Rec 5 line is progressing less quickly as the recombinant chromosome is not transmitted to 50% of the offspring. We currently have 2 Rec5 males who are being mated to generate Rec5 females. The Rec6 mice were identified only recently and we have one male who is being crossed with B6 females to generate recombinant females.

Task 3: Production of female *Min*/+ mice carrying the recombinant chromosome. Months 5-24; Mice: 200 recombinant females (from Task 2), about 75 *Min*/+ males, about 800 *Min*/+ females weaned and genotyped for chromosome 6.

Progress: Females heterozygous for the recombinant chromosomes were crossed with B6 *Min*/+ males. The resulting female progeny were genotyped at *Apc* to identify the *Min*/+ females. The *Min*/+ females were

then genotyped for markers on chromosome 6. The goal was to identify about 20 *Min/+* females carrying each recombinant chromosome and 20 of their siblings who carried B6 alleles throughout the region. This has been accomplished for lines 1, 2 and 3. We are currently crossing 4 Rec 4 females with *Min/+* males to generate females to test. We have 3 Rec6 females being crossed with *Min/+* males.

Task 4: Testing of females carrying recombinant chromosomes for modifier phenotype. Months 6-30; Mice: The 800 females produced in Task 3.

Progress: The females from lines 1,2 and 3 produced in Task 3 have been treated with ENU, and tested for mammary tumor development. The results are shown in Table 1 below . For lines 1 and 3, neither the number or tumors (by Wilcoxon Rank sum test) nor the tumor incidence (by chi square) was different for mice carrying the congenic recombinant chromosome and those carrying only B6 alleles in the region. For the Rec2 line, the both the number of tumors (p=0.0002, Wilcoxon Rank sum test) and the number of tumor bearing mice (0=0.0002 Chi square), was different in the mice carrying the congenic recombinant chromosome as compared with those carrying only B6 alleles in the region.

Table 1. Mammary tumor susceptibility in R26 recombinant lines.

Markers	**Rec1**		**Rec3**		**Rec2**	
Mit3	129	B6	129	B6	B6	B6
Mit36	129	B6	129	B6	B6	B6
Mit106	129	B6	B6	B6	B6	B6
R26	+	+	+	+	R26	+
Mit55	B6	B6	B6	B6	129	B6
Tumor #	3.2	3.5	2.1	2.8	0.11	2.8
# of mice	12	13	16	13	9	12
% with tumors	92	100	94	100	11	92

These results indicate that we have localized a modifier of mammary tumor susceptibility to a region of about 3 cM around the ROSA26 locus. Mice that we have on hand, the Rec5 and Rec6 mice, should allow us to further limit the interval within the next 6 months.

Task 5: Establishment of lines carrying recombinant chromosomes to allow further testing. Each recombinant chromosome will be replicated and maintained by intercrossing mice heterozygous for the recombinant chromosome and selecting for mice homozygous, thus preserving the recombinant region intact. Months 4-36; Mice: About 4 cages/per line, or about 48 mice /line/year.

Progress: We have established mice that are homozygous for Rec1 and 3. These lines are currently being maintained. We have tried without success to obtain mice homozygous for the congenic region carried by the Rec2 line. We are maintaining this line in the heterozygous state. We also have had difficulty in establishing a line of mice carrying the original ROSA26 congenic interval. Homozygous mice are obtained at much less than the expected 25% and many homozygotes identified are runted and fail to breed. The exact reason for his difficulty is unknown although a likely explanation is that the locus (loci) affected by ROSA26 insertion is required for normal growth and development.

Task 6: Identification and testing of candidate loci by identification of ESTs in the region and mapping using recombinant chromosomes. Months 10-36, no mice.

Progress: None

Task 7. Production and testing of mice transgenic for candidate modifier genes. This Task will be dependent on the progress made in Tasks 1-5. If the number of candidates gets down to 1-2, they will be tested for function as transgenes.

Progress: None

Aim 2.

Task 1: Testing tumors for allele loss at *Apc*. Months 1-6, no mice, this will utilize archived tumor samples.

Progress: We have not yet tested any tumors for LOH. The UW Comprehensive Cancer Center and the Environmental Health Sciences Center have jointly established a Laser Capture Microscopy Facility. We have access to the facility and are beginning to collect samples from tumors for analysis. Careful histological analysis of the mammary tumors indicated that without a laser capture device it would be difficult to obtain samples that were mainly tumor tissue and did not include large amounts of normal tissue. By using Laser Capture, we can obtain smaller samples from numerous sites within the tumor an decrease the amount of normal tissue contamination.

Task 2: Testing tumors for allele loss in modifier region. Months 1-36, no mice. As the modifier interval is narrowing by analysis of the modifier region, we can test more directly for loss of the modifier locus.

Progress: See Task 1.

Task 3. Compilation of results and preparation of publication. Months 6-8, 34-36.

Progress: We are currently preparing a manuscript describing the effect of ROSA26 on mammary tumor susceptibility and the analysis of the Rec1, 2 and 3 lines.

KEY RESEARCH ACCOMPLISHMENTS

- We have identified 12 markers on chromosome 6 that are polymorphic between B6 and 129. We have determined the map location of these markers. In some cases, the map position differs from the published order.
- We have characterized and generated 6 congenic lines carrying a region of 129-derived DNA on the B6 background.
- We have localized a modifier of *Min*-induced mammary tumor susceptibility to a region of about 3 cM on chromosome 6.

REPORTABLE OUTCOMES

Presentations: Genetic Analysis of Mammary Tumor Susceptibility in *Min*/+ Mice, at "Genetics, Genomics and Molecules, Madison, WI, 5/23-25/99.

Manuscripts: Kohlhepp, RL, Nett, J, Hegge, LF, and Moser, AR. "Genetic Analysis of a Mammary Modifier Locus in ROSA26 Mice", In prepartion.

CONCLUSIONS

We have identified 6 mice carrying recombinant chromosomes from the congenic region carried by the B6.ROSA26 mice. We have established lines form each of these mice and have tested three of the chromosomes for the effect on mammary tumor development in *Min*/+ mice. Three other lines are currently being expanded and tested. The results of the mammary tumor studies results indicate that we have localized a modifier of mammary tumor susceptibility to a region of about 3 cM around the ROSA26 locus. This congenic chromosome is carried by mice of the Rec2 line. Mice that we have on hand, the Rec5 and Rec6 mice, should allow us to further limit the interval within the next 6 months.

The localization of the modifier within this 2-3 cM region is the first step toward the molecular identification of the locus. The fact that we have been as yet unable to separate the modifier from the ROSA26 insertion site increases the likelihood that the insertion is responsible for the phenotype. In order to try and collect more evidence for a modifier in this region, we are in the process of generating congenic lines carrying 129-derived DNA in this region, but lacking the ROSA26 insertion. We are generating at least three congenic lines and will be able to test these lines over the next year. It is also possible that there is a modifier and that the ROSA26 insertion also has an effect. These congenic mice should help to discriminate between these options. Therefore, while we will continue to try and collect further recombinants from the ROSA26 mice, we will also test the congenic mice not carrying ROSA26.

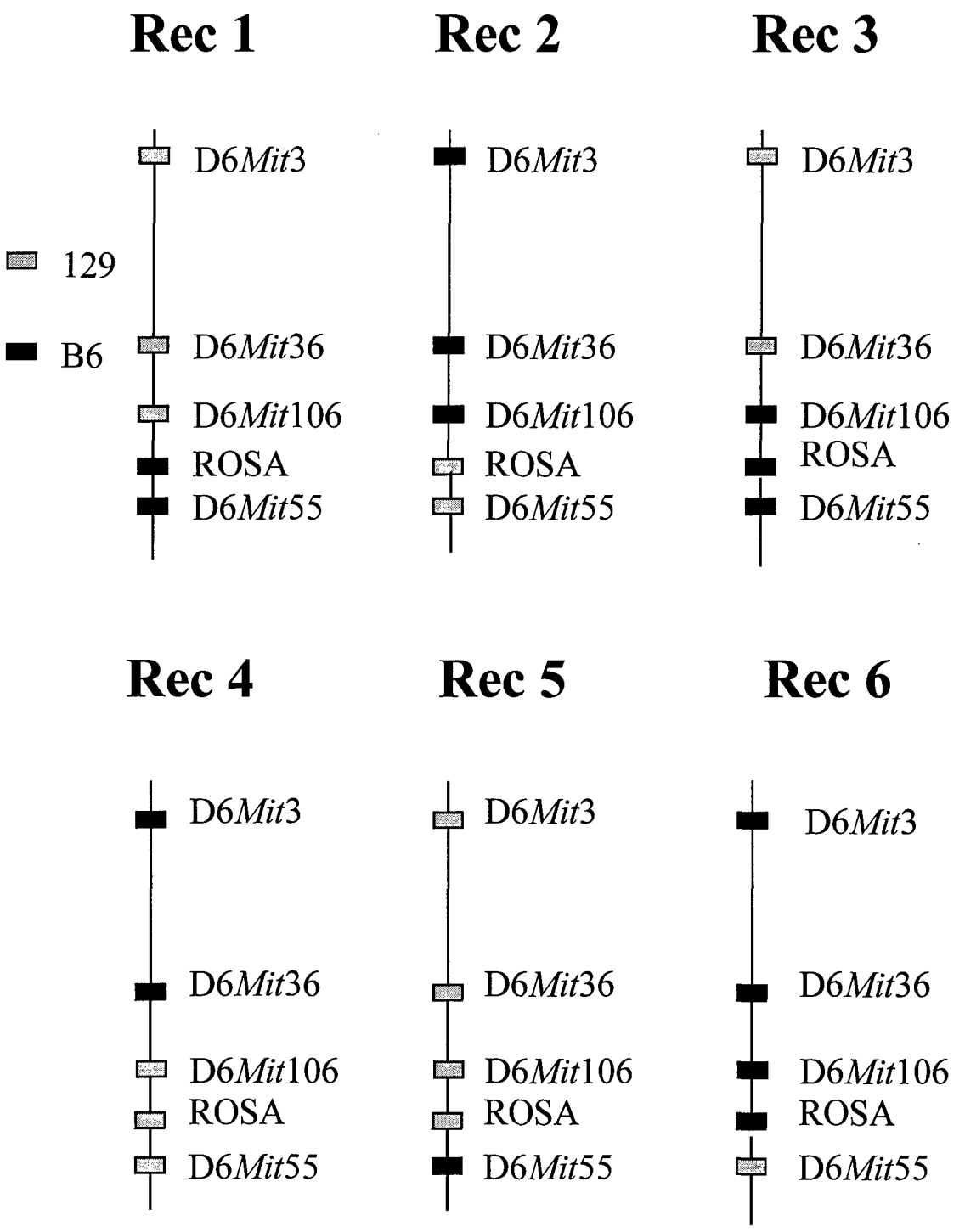
REFERENCES

None

APPENDICES

Attached: Figure 1.

Figure 1.
Recombinant chromosomes identified from the R26 mice



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